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INDUCED MATURATION AND SPAWNING OF MILKFISH, *CHANOS CHANOS* FORSSKAL, BY HORMONE IMPLANTATION

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ABSTRACT

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The milkfish, *Chanos chanos* Forsskal, does not reach gonadal maturity easily in captivity. In an attempt to induce maturation, exogenous hormones, LHRH-A and 17α-methyl-testosterone, were implanted into adult milkfish either alone or in combination. The hormones were delivered using cholesterol pellets (LHRH-A) or silastic tubing sealed with elastomer (17α-methyl-testosterone). The fish were implanted three times at monthly intervals between March and May of 1985. The combination of LHRH-A and 17α-methyl-testosterone induced significantly more maturing fish ($P < 0.05$) than LHRH-A alone or sham controls: 88%, 38%, and 13%, respectively.

Fish with average egg diameters between 768 μm and 905 μm, spawned 48 h after hormone implantation. These results indicate that the maturation and spawning of milkfish in tanks can be induced and accelerated 1—2 months earlier than the beginning of the normal spawning season through hormone implantation.

INTRODUCTION

During the past decade, there have been many attempts to spawn milkfish (*Chanos chanos* Forsskal) in captivity. Artificial induction of milkfish spawning has been achieved in the Philippines (Juario et al., 1984), Taiwan (Liao and Chen, 1984; Lin, 1984) and Hawaii (Lee and Weber, 1983). Natural spawning of milkfish has also been achieved in ponds (Lin, 1984) and sea cages (Marte et al., 1983). From these reports, it is clear that major constraints to the development of mass artificial propagation of milkfish include the limited resource of mature broodstock and stress from frequent handling.

The injection of exogenous gonadotropins has been used traditionally to induce the final maturation of captive female fish at the completion of vitellogenesis. In captivity, milkfish usually do not reach this stage of ovarian development so that a greater frequency of hormone treatments has been

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applied to induce maturation. Multiple injections result in handling stress which negates the potency of applied exogenous hormones because stress blocks the normal reproductive cycle in fish (Billard et al., 1981). A technique which delivers the required hormone and reduces handling stress is therefore extremely desirable. Various alternative methods of chronic hormone administration (i.e., incorporation of hormones into the diet, Freund's adjuvant, moltant cocoa butter, etc.) have been employed in other fish to reduce the frequency of injection and stress (Crim, 1985).

This paper reports trials with a new LHRH-A cholesterol pellet, prepared at the Oceanic Institute in Hawaii, to determine its ability to induce the maturation and spawning of captive milkfish.

MATERIALS AND METHODS

Synthetic des-Gly^{10}-(D-Ala^{6})-LHRH ethylamide analogue (abbreviated as LHRH-A), and 17α-methyl-testosterone were purchased from Sigma Chemical Co., U.S.A. LHRH-A was incorporated into cholesterol pellets that contained 200 μg of LHRH-A. These pellets were prepared by first dissolving 2 mg of LHRH-A in 0.3 ml of 50% ethanol. The solution was then mixed with 190 mg of cholesterol (USP grade) until a paste-like consistency was obtained. The paste was dried in an incubator set at 35°C, and the resulting dried powder was mixed with 10 mg of cocoa butter which served as a binder. Finally, the mixture was packed into a pellet using a plexiglass mold. The resulting pellet weighs approximately 23 mg and has an average length of 5.5 mm and diameter of 2.4 mm. A procedural guide to the production and implantation of the LHRH-A cholesterol pellet into milkfish is presented elsewhere (Lee et al., 1985). The 17α-methyl-testosterone was dissolved in castor oil and placed into 2 cm pieces of silastic tubing sealed with medical grade elastomer. The silastic tubing contained 250 μg of 17α-methyl-testosterone.

Milkfish do not exhibit any sexual dimorphism and thus adult milkfish (6—8 years old) were randomly stocked into four round fiberglass tanks (6 m in diameter) with an assumed sex ratio of 1:1. Ten fish were placed in each tank. In each of two tanks, designated Group 1, eight fish received intramuscular implants of LHRH-A and 17α-methyl-testosterone using the procedure described by Lee et al. (1985). The two remaining individuals in each tank were implanted with placebos and served as sham controls. The fish in the remaining two tanks were treated similarly except that these fish only received the LHRH-A alone and were designated as Group 2.

Fourteen fish denoted as Group 3 were stocked in a rectangular tank measuring 6.7 × 6.5 × 1.3 m. Ten were implanted with LHRH-A cholesterol pellets and the other four fish were implanted with placebos and served as sham controls. Twenty-four fish denoted as Group 4 were placed in another rectangular tank of the same size. These fish were not implanted at all and served as controls for Group 3.

At the time of implantation and on a monthly basis, each fish was checked for the presence of oocytes or sperm using the cannulation technique described by Shehadeh et al. (1973). Egg diameters were measured under a light microscope to 0.05 mm. Spawned eggs were collected and their diameters were similarly measured. Those fish receiving LHRH-A pellet implantations were re-implanted, once each month.

Fish in each tank were fed Purina trout chow (32% crude protein) at 1% of their body weight twice a day. Water temperature, salinity and turbidity were monitored at the time of feeding. Constant water flow rate was maintained at an exchange rate of about 25% of total water volume daily. Tanks were aerated by two large airstones.

RESULTS AND DISCUSSION

Water temperature during this experiment ranged from 24°C to 26°C and salinity ranged from 33 to 37 ppt. There was no difference in these measurements among the experimental groups.

The criterion for maturing males was whether sperm could be expelled from the urogenital pore with slight pressure exerted by stroking the abdomen. At the end of the second month, 13 treated fish were identified as maturing males; nine in Group 1 receiving LHRH-A and 17α-methyl-testosterone, three in Group 2 receiving LHRH-A alone, and one hormone-implanted fish from Group 3 (Table 1). There was one maturing male among the sham control of Group 1 and none from control Group 4.

TABLE 1

Number of maturing fish in different groups

Group no.	Treatment	Maturing		Not maturing	Total	% of maturing fish
		Female	Male			
1	LHRH-A and testosterone	5	9	2	16	88
	Sham control	0	1	3	4	25
2	LHRH-A	3	3	10	16	38
	Sham control	0	0	4	4	0
3	LHRH-A	2	1	7	10	30
	Sham control	0	0	4	4	0
4	Control	0	0	24	24	0

In females, an individual found to possess vitellogenic ova with an average egg diameter of 700 μm or more was classified as mature. This is the minimum egg diameter in which the final stage of oocyte maturation (i.e., hydration) has been successfully induced by use of exogenous gonadotropins (Kuo, 1985). There were five fish in Group 1, three fish in Group 2

TABLE 2

Date of implantation and spawning (data in parentheses give egg size in mm ± SD)

Fish No.	Treatment	Hormone implantation			Spawning	
		1st	2nd	3rd	1st	2nd
1	LHRH-A and liquid testosterone	7 Mar. 85 (NA)	9 Apr. 85 (NA)	7 May 85 (0.768±0.044)	9 May 85 (1.220±0.042)	
2	LHRH-A and liquid testosterone	14 Mar. 85 (NA)	11 Apr. 85 (0.435±0.042)	10 May 85 (0.857±0.051)	12 May 85 (1.290±0.042)	
3	LHRH-A and liquid testosterone	14 Mar. 85 (NA)	11 Apr. 85 (0.790±0.029)	10 May 85 (0.583±0.075)	13 Apr. 85 (1.269±0.027)	
4	LHRH-A	7 Mar. 85 (NA)	9 Apr. 85 (0.887±0.071)	2 May 85 (0.317±0.037)	11 Apr. 85 (1.284±0.038)	
5	LHRH-A	14 Mar. 85 (NA)	11 Apr. 85 (0.853±0.032)	9 Mar. 85 (0.634±0.078)	13 Apr. 85 (1.204±0.037)	
6	LHRH-A	28 Feb. 85 (NA)	15 Apr. 85 (0.375±NA)	6 May 85 (0.905±0.066)	26 Mar. 85 (NA)	8 May 85 (1.345±0.049)

NA: Data not available.

and two fish in Group 3 that were classified as mature. No maturing females were found in the sham treated or controls. The holding tanks for Group 2 and Group 3 were different in shape, but the percentage of individuals showing signs of maturation in both groups was about the same.

Spawnings were observed following the first hormone implantation (Table 2). Fish with average egg diameters of 768—905 μm responded to the hormone implantation and spawned 48 h after implantation. Fish no. 6, with an unknown egg diameter at first implantation, spawned shortly before the second implantation. The same fish (no. 6) had eggs with an average diameter of 905 μm at the time of third implantation. She spawned again 2 days later, indicating that milkfish are capable of multiple spawnings in a single season. This conclusion is contrary to the proposal that milkfish have a long reproductive cycle of more than 1 year (Lacanilao et al., 1984; Lam, 1984).

In this experiment, milkfish spawned in the months of April and May. This is 1—2 months earlier than the beginning of the normal spawning season in Hawaii, June—August (Kuo and Nash, 1979). Therefore, maturation of milkfish was accelerated by the implantation of hormones. Ovulation of landlocked Atlantic salmon (*Salmo salar*) was also accelerated by intraperitoneal implantation of LHRH-A pellets (Crim et al., 1983).

Fertilization of spawned eggs was not achieved in most of the spawnings due to an absence of mature males in the tank at the time of spawning. However, one spawning (fish no. 3) achieved 95% fertilization. Fish nos. 1 and 2 spawned in tanks with mature males but no fertilization was observed. There is no explanation for these results at this time.

Although the results are preliminary, there appears to be some promise in the use of the pelleted hormones in enhancing the maturation of gametes in milkfish. A thorough description of ongoing experiments focused on the maturation of milkfish is to be presented in the future. However, the superior performance of the LHRH-A cholesterol pellet in combination with the 17α-methyl-testosterone silastic tubing pellet in producing a significant larger number of maturing individuals should be noted. The apparent success of this treatment may hinge on two factors: (1) the use of hormone pellet technology which delivers the desired chemical messenger over extended periods of time and thus reduces the amount of physical handling, and (2) the choice of hormones used. 17α-methyl-testosterone has been reported to stimulate gonadotropin synthesis in the pituitary (Crim and Evans, 1979, 1982, 1983). The function of the LHRH-A molecule is to govern the release of the native gonadotropin from the pituitary. The coupling of the hormones used and the vehicles by which they are administered may be providing a continuous stimulus for maturation of the gonads in the form of a sustained release of native gonadotropin from the pituitary.

In summary, 14 (five females and nine males) of the 16 fish that received LHRH-A and 17α-methyl-testosterone showed maturing or mature

gonads. Nine (five females and four males) of the 26 fish showed maturation of gonads after they were implanted three times with LHRH-A pellets alone. Thus, twice as many fish matured in the treatment with the combination of 17α-methyl-testosterone and LHRH-A than in the treatment with LHRH-A pellets alone. The percentage of mature fish is highly dependent on the treatment ($P < 0.05$) by G-test (Sokal and Rohlf, 1969). These results indicate that hormone implantation shows promise for controlling reproduction in milkfish. However, further characterization of the hormone pellets' action is essential and is ongoing.

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